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EXHIBIT A

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PATENT
Attorney Docket No. MERCK-2056



**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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| APPLICANT(S): | Hartmann *et al.* | CONF. NO.: | 2626 |
| SERIAL NO.: | 09/708,506 | GROUP NO.: | 1647 |
| FILING DATE: | November 9, 2000 | EXAMINER: | DeBerry, Regina M. |
| TITLE: | Erythropoietin Forms with Improved Properties | | |

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

## AMENDMENT AND RESPONSE

Sir:

The Office action of January 6, 2004, rejected claims 1-5, 10, 11, 13, 16-18, and 24-29. Reconsideration and withdrawal of the rejections is respectfully requested in view of the following amendments and remarks.

Applicants enclose a petition and fee for a three-month extension of time to July 6, 2004, for responding to the Office Action. Applicants believe that no other fees are due with this submission. Nevertheless, please consider this a conditional authorization to charge any additional fees to Deposit Account No. 20-0531.

**Amendments to the Specification** begin on page 2 of this paper.

**Amendments to the Claims** are reflected in the listing of claims, which begins on page 3 of this paper.

**Remarks** begin on page 7 of this paper.

## AMENDMENTS TO THE SPECIFICATION

On page 37, please replace the paragraph starting with "For example" and ending with "amino acids alone" with the following amended paragraphs:

For example, the following set of experiments with controls is performed. Human Fc-EPO, human Fc-EPO ($Cys_{29}$-$Cys_{88}$), human EPO, and human EPO ($Cys_{29}$-$Cys_{88}$) are cleaved with trypsin in both reducing and non-reducing conditions. These eight samples are analyzed by mass spectrometry. Trypsinized non-reduced human Fc-EPO ($Cys_{29}$-$Cys_{88}$) and human EPO ($Cys_{29}$-$Cys_{88}$) each give a peak with a high molecular weight, corresponding to EAENITTGCAEGPSLNENITVPDTK (SEQ ID NO:28) + GQALLVNSSQPCEPALQLHVDK (SEQ ID NO:29) with two N-linked glycosylations. Because of its large size and heterogeneity due to the presence of two N-glycosylations, this peak is easily distinguished from the other peaks. This peak is not found in reduced samples or in samples derived from non-mutant human EPO or non-mutant human Fc-EPO. As a further diagnostic test, samples are incubated with N-glycanase before treatment with trypsin.

In the samples treated with N-glycanase, the peak corresponding to EAENITTGCAEGPSLNENITVPDTK (SEQ ID NO:28, corresponding to pos. 21 – 45, SEQ ID NO:2) +
GQALLVNSSQPCEPALQLHVDK (SEQ ID NO:29, corresponding to pos. 77 – 97, SEQ ID NO:2) is shifted to the size predicted by the molecular weights of the amino acids alone.

## AMENDMENTS TO THE CLAIMS

Please amend the claims as follows:

1. (Canceled)

2. (Currently amended) The fusion protein ~~An EPO form~~ of claim 28 having ~~1, showing~~ improved biological activity compared to naturally-occurring human erythropoietin.

3. (Currently amended) The ~~A~~ fusion protein of claim 28 ~~1~~ having an extended serum half-life compared to naturally-occurring human erythropoietin.

4. (Currently amended) The ~~A~~ fusion protein of claim 3, wherein said extended serum half-life is greater than 20 hours.

5. (Currently amended) The fusion protein~~s (iii), (iv), (vi) or (vii)~~ of claim 30 ~~1~~, ~~wherein said fusion proteins~~ having ~~have~~ greater specific activity than the comparable Fc-EPO fusion proteins having no mutations in the EPO portion ~~mutated EPO molecules~~.

6-15. (Canceled)

16. (Currently amended) The ~~A~~ fusion protein of ~~according to~~ claim 30 ~~1, said fusion protein~~ comprising a whole Ig molecule.

17. (Currently amended) The ~~A~~ fusion protein of ~~according to~~ claim 30 ~~1,~~ wherein the ~~Ig molecule~~ Fc portion and the EPO portion ~~molecule~~ are of mammalian origin.

18. (Currently amended) The ~~A~~ fusion protein of ~~according to~~ claim 17, wherein the Fc portion is derived from ~~Ig molecule is~~ human IgG.

19-23. (Canceled)

24. (Currently amended) A pharmaceutical composition comprising the ~~a~~ fusion protein of ~~according to~~ claim 30 ~~1~~ and a pharmaceutically acceptable carrier, diluent or excipient.

25. (Currently amended) The ~~A~~ pharmaceutical composition of claim 24 containing at least one additional pharmaceutically effective drug and / or adjuvants.

26. (Canceled)

27. (Currently amended) The ~~A~~ fusion protein of claim 32 ~~26~~, wherein the EPO portion comprises ~~EPO$_m$ is derived from human EPO and has~~ at least one of the following mutations: $His_{32}$→Gly, $Ser_{34}$→Arg, and $Pro_{90}$→Ala.

28. (Currently amended) The ~~A~~ fusion protein of claim 33 ~~26~~, wherein the EPO portion ~~EPO$_m$~~ comprises cysteines at positions 29 and 88.

29. (Currently amended) The ~~A~~ fusion protein of claim 28 ~~26~~, wherein the EPO portion ~~EPO$_m$~~ comprises cysteines at positions 29, 33, 88, and 139.

30. (New) A fusion protein comprising an Fc portion of an Ig molecule and an erythropoietin (EPO) portion, wherein (i) the Fc portion is fused covalently via its C-terminus directly or indirectly to the EPO portion, (ii) the EPO portion comprises a Cys substitution at an amino acid position corresponding to $Gln_{86}$, $Pro_{87}$, $Trp_{88}$, $Glu_{89}$, or $Leu_{91}$ of human erythropoietin, and (iii) the EPO portion retains erythropoietin activity.

31. (New) The fusion protein of claim 30, wherein the EPO portion comprises an amino acid other than cysteine at position 33.

32. (New) The fusion protein of claim 30, wherein the EPO portion is derived from human erythropoietin.

33. (New) The fusion protein of claim 32, wherein the EPO portion comprises Cys at position 88.

34. (New) The fusion protein of claim 33, wherein the EPO portion further comprises at least one of the following amino acid variations: position 29 is not Cys, position 33 is not Cys, and position 139 is Cys.

35. (New) The fusion protein of claim 30, wherein the Fc portion is mutated or truncated in its amino acid sequence.

36. (New) The fusion protein of claim 30, wherein the Fc portion is modified in its glycosylation pattern.

37. (New) The fusion protein of claim 30, wherein the Fc portion is derived from an IgG chain and comprises a mutation of the glycosylation site within the Fc portion of the IgG chain.

38. (New) The fusion protein of claim 37, wherein the mutation is of an asparagine at an amino acid position corresponding to position 297 of IgG1.

39. (New) The fusion protein of claim 30 further comprising a linker between the Fc portion and the EPO portion.

40. (New) The fusion protein of claim 39, wherein the linker has no protease cleavage site.

## REMARKS

Claims 1-5, 10, 11, 13, 16-18, and 24-29 were pending in this application. Claims 8-9 were withdrawn from consideration as being directed to non-elected species. Claims 1, 8, 9, 11, 13, and 26 are now cancelled without prejudice to Applicants' right to prosecute their subject matter in the present application and in related applications. New claims 30-40 are added and claims 2-5, 16-18, 24, 25, and 27-29 are currently amended without any intent of disclaiming equivalents thereof. Accordingly, claims 2-5, 16-18, 24, 25, and 27-40 are pending and presented for consideration.

*Specification amendments*

Applicants have amended the specification to correct a typographical error. Support for the amendment is found in the specification at least, for example, at page 35, lines 10-12, and at page 21, lines 26-32. Applicants respectfully submit the amendment to the specification introduces no new matter.

*Claim amendments*

Support for the claim amendments can be found in the specification, including the claims as originally filed. Support for the recitation of "compared to naturally-occurring human erythropoietin" in amended claims 2 and 3 is found in the specification at least, for example, at page 15, lines 15-19. Support for the recitation of "mutations in the EPO portion" in amended claim 5 is found in the specification at least, for example, at page 6, lines 14-15. Support for the recitation of "Fc portion is derived from" in amended claim 18 is found in the specification at least, for example, at page 25, lines 10-11. Claims 2, 5, 16, and 27 have been amended to delete unnecessary words. Claims 2-5, 16-18, 24, and 27-29 have also been amended for consistency with independent claim 30 from which they depend and to correct dependencies.

Support for new claim 30 is found in the specification at least, for example, at page 6, lines 11-14, page 9, lines 24-25 and 31-33, and page 13, lines 12-14. Support for new claim 31 is found in the specification at least, for example, at page 13, lines 12-14. Support for new claim

32 is found in the specification at least, for example, at page 6, line 28. Support for new claim 33 is found in the specification at least, for example, at page 13, line 13. Support for new claim 34 is found in the specification at least, for example, at page 12, lines 31-34. Support for new claim 35 is found in the specification at least, for example, at page 9, lines 1-2. Support for new claim 36 is found in the specification at least, for example, at page 9, line 2. Support for new claim 37 is found in the specification at least, for example, at page 8, lines 31-32. Support for new claim 38 is found in the specification at least, for example, at page 9, line 16. Support for new claim 39 is found in the specification at least, for example, at page 10, lines 31-32. Support for new claim 40 is found in the specification at least, for example, at page 11, lines 4-5.

Applicants submit that these amendments introduce no new matter.

*Personal interview*

Applicants thank Examiner Regina M. DeBerry and Examiner Yvonne Eyler for the personal interview on March 15, 2004, discussing the outstanding rejections in this patent application. Applicants have attempted to incorporate the substance of several of the Examiners' suggestions, provided in the Office action and during the interview, into the present paper. The following comments address the claim objection and rejections in the order that they were raised in the Office action.

*Claim Rejections Under 35 U.S.C. § 112, first paragraph, written description*

Claims 1 and 26 presently stand rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. Claims 1 and 26 are cancelled without prejudice and without acquiescing to the rejections thereto. Applicants therefore submit that the rejections under 35 U.S.C. § 112, first paragraph, are moot.

*Objection to Specification*

The Office action objects to the amendment filed on September 26, 2003, alleging that it introduces new matter into the specification. The allegedly new material, "Fcm-L-EPOm" and "EPOm is EPO which is mutated in its amino acid sequence and comprises at least one of the following changes," was recited in claims 1 and 26. Claims 1 and 26 are cancelled without

prejudice and without acquiescing to the rejections thereto. Applicants therefore request reconsideration and withdrawal of the objection.

*Claim Rejections Under 35 U.S.C. § 112, first paragraph, scope of enablement*

Claims 1-5, 10-13, 16-18, and 24-29 stand rejected under 35 U.S.C. § 112, first paragraph, on the grounds that the scope of the claims is allegedly not commensurate with the scope of the enabling disclosure. In particular, the Office action alleges that the specification, while being enabling for an Fc-$EPO_m$ fusion protein wherein the $EPO_m$ is derived from human EPO and has the following mutations: $His_{32}$→Gly, $Cys_{33}$→Pro and $Trp_{88}$→Cys, does not reasonably provide enablement for an Fc-$EPO_m$ fusion protein wherein the $EPO_m$ comprises at least one of the following changes: $Asn_{24, 38, 83}$→Gln, $Ser_{126}$→Ala, $His_{32}$→Gly, $Ser_{34}$→Arg and $Pro_{90}$→Ala (claim 1) or wherein the $EPO_m$ comprises Cys at position 88 and at least one of the following amino acid variations: position 29 is not Cys, position 33 is not Cys, and position 139 is Cys (claim 26) or wherein the $EPO_m$ is derived from human EPO and has at least one of the following mutations: $His_{32}$→Gly, $Ser_{34}$→Arg and $Pro_{90}$→Ala (claim 27) or wherein the $EPO_m$ comprises cysteines at positions 29 and 88 (claim 28) or wherein the $EPO_m$ comprises cysteines at positions 29, 33, 88, and 139 (claim 29). The Office action further alleges that "[t]he instant specification could not support claims to EPO polypeptides modified to an unlimited extent," that it is unpredictable how the mutations or deletions as recited in the instant claims would affect Fc-EPO protein activity; and that "the changes which can be made in the structure and still maintain sufficient activity" are unpredictable "and the experimentation left to those skilled in the art is unnecessarily and improperly extensive and undue." Applicants traverse this rejection to the extent it is maintained over the pending claims.

The test for enablement is whether one reasonably skilled in the art could make or use the invention as broadly as it is claimed based on the disclosures in the specification coupled with information known in the art without undue experimentation. *See In re Wands*, 858 F.2d 731 (CAFC 1988); *Ex parte Mark*, 12 USPQ2d 1904 (BPAI 1989). In *Wands*, the court faced the question whether the specification of the Wands patent enabled one skilled in the art to make high affinity IgM monoclonal antibodies for hepatitis B-surface antigen. The *Wands* court

recognized that the nature of monoclonal antibody technology involved screening hybridomas to determine which ones secrete antibodies with desired characteristics. *Id.* at 740. The court stated: "Enablement is not precluded by the necessity for some experimentation such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation. 'The key word is 'undue,' not 'experimentation.'" *Id.* at 736-737. "The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." *Id.* at 737. In deciding whether undue experimentation is involved for practicing the invention as claimed, the court considered the following eight factors: "(1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability of the art, and (8) the breadth of the claims." *Id.* at 737. The court concluded that undue experimentation would not be required to practice the invention because (1) Wands' disclosure provided considerable direction and guidance on how to practice their invention and presented working examples, (2) there was a high level of skill in the art at the time when the application was filed, and (3) all of the methods needed to practice the invention were well known. *Id.* at 737, 740.

Consistent with the reasoning and the outcome in *Wands*, the Board of Patent Appeals and Interference, in *Ex parte Mark*, found a claim reciting "a synthetic mutein of a biologically active native protein in which native protein has at least one cysteine residue that is free to form a disulfide link and is nonessential to said biological activity, said mutein having at least one of said cysteine residues substituted by another amino acid and said mutein exhibiting the biological activity of said native protein" enabled by the specification because, "for a given protein having cysteine residues, one skilled in the art would be able to routinely determine whether deletion or replacement of the cysteine residues would result in a mutein which is within the scope of the claims." *Mark*, 12 USPQ2d 1904.

Applying this test to the instant application, Applicants submit the specification fully enables the invention as claimed in the pending claims. First, Applicants submit that, like in

*Wands* and *Mark*, the specification and claims of the instant application provide reasonable guidance or directions on how to practice the invention as claimed in the pending claims. Specifically, the specification provides adequate guidance on (1) how to generate cysteine substitutions and other mutations in Fc-EPO and (2) how to screen and select for mutated Fc-EPO proteins with desired functional characteristics. For example, from page 13, line 4, to page 15, line 28, the specification provides detailed guidance on how to design and generate altered disulfide bonds. From page 29, line 31, to page 30, line 17, and from page 30, line 30, to page 31, line 18, the specification provides detailed guidance on how to generate mutant forms of Fc-EPO by site-directed mutagenesis and random mutagenesis methods. On page 29, lines 1-29, and page 30, lines 20-27, the specification provides detailed guidance on how to test the *in vitro* activity of mutant forms of Fc-EPO. From page 31, line 21, to page 32, line 22, the specification provides detailed guidance on how to perform pharmacokinetic assays to test Fc-EPO proteins for desired serum half-life. From page 32, line 25, to page 35, line 4, the specification provides detailed guidance on how to test Fc-EPO fusion proteins for desired *in vivo* activity. Furthermore, as the Examiner acknowledged, example 13 of the specification provides a detailed working example of the claimed invention.

Secondly, Applicants submit that, like in *Wands*, where there was a high level of skill in the art of monoclonal antibody generation at the time when the application was filed, there was a high level of skill in the art of EPO structure and function when this application was filed. Specifically, the EPO structure-function relationship had been well studied at the time of filing, permitting prediction of the effects of certain amino acid changes on EPO function based on the well-characterized EPO protein structure. For example, Boissel *et al.* proposed a structure model of EPO predicting a four alpha-helical bundle motif based on its primary sequence and the location of its disulfide bonds. (Boissel *et al.*, 1993, Erythropoietin Structure-Function Relationships: Mutant Proteins That Test a Model of Tertiary Structure, J. Biol. Chem., 268(21):15983-15993, a copy of which is enclosed as Exhibit A). This model was tested by site-directed mutants and deletion mutants. *Id.* The study revealed that the predicted alpha-helices were not likely to tolerate amino acid changes, while the N-terminus, the C-terminus, and the interhelical loops can tolerate small deletions and point mutations. *Id.* Elliott *et al.* also used

epitope mapping to suggest a model of recombinant human erythropoietin structure that is consistent with a structure of 4-helix bundle with short and long interconnecting loops (Elliott *et al.*, 1995, Fine-Structure Epitope Mapping of Antierythropoietin Monoclonal Antibodies Reveals a Model of Recombinant Human Erythropoietin Structure, Blood, 87(7):2702-2713, a copy of which is enclosed as Exhibit B). Amino acid sequences of mature EPO from different mammals had been compared to identify highly conserved regions and relatively variable regions (Wen *et al.*, 1993, Erythropoietin structure-function relationships: high degree of sequence homology among mammals, Blood, 82(5):1507-16, a copy of which is enclosed as Exhibit C). The highly conserved regions included (1) the disulfide bridge linking the $NH_2$ and COOH termini; (2) N-glycosylation sites; and (3) predicted amphipathic alpha-helices. *Id.* In contrast, the short disulfide bridge between cysteine 29 and cysteine 33 was not conserved. *Id.* Other variable regions included the C-terminal part of the loop between the helices C and D. *Id.* Furthermore, Wen *et al.* identified functionally important domains on the surface of the EPO molecule (Wen *et al.*, 1994, Erythropoietin Structure-Function Relationships, J. Biol. Chem. 269(36):22839-22846, a copy of which is enclosed as Exhibit D). Elliott *et al.* mapped active sites of EPO and identified four regions that are important for EPO biological activity (*e.g.*, Elliott *et al.*, 1997, Mapping of the Active Site of Recombinant Human Erythropoietin, Blood, 89(2):493-502, a copy of which is enclosed as Exhibit E). Finally, as disclosed at page 2, lines 8-10, of the specification, Syed *et al.* determined the crystal structure of human EPO complexed to the extracellular ligand-binding domains of EPO receptor, providing a detailed picture of the amino acids at the interfaces between EPO and its receptor (Syed *et al.*, 1998, Efficiency of Signaling through Cytokine Receptors Depends Critically on Receptor Orientation, Nature, 395:511-516, a copy of which is enclosed as Exhibit F). Thus, contrary to the Examiner's assertion that it was in no way predictable how mutations or deletions would affect EPO activity, Applicants submit that, based on (1) the known active sites and domains for EPO function, (2) the well-characterized conserved regions and relatively variable regions, and (3) the high-resolution crystal structure of human EPO, there was a high level of skill in the art providing significant guidance regarding amino acid positions that were or were not likely to tolerate changes.

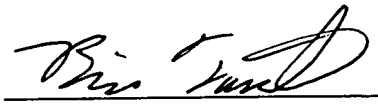
Thirdly, Applicants submit that, like in *Wands*, where methods needed to practice the invention were known in the art, all of the methods needed to generate Fc-EPO mutation and to test Fc-EPO function and serum half-life were well known in the art when the application was filed. As discussed above, assays for testing EPO *in vivo* and *in vitro* biological activities and for testing serum half-life are described in the specification. Additional *in vitro* and *in vivo* activity assays were described in PCT publication WO 99/02709, filed on July 6, 1998 and published on January 21, 1999, a copy of which is enclosed as Exhibit G (*see, e.g.*, page 28, line 6 to page 29, line 7 of Exhibit G). Additional *in vitro* assays using three different EPO-responsive target cells were also disclosed at page 22840 of Exhibit D. Additional assays for measuring Fc-EPO serum half-life were described from page 19, lines 22, to page 20, line 5 of Exhibit G. Therefore, Applicants submit that, like in *Mark*, for a given Fc-EPO mutant, one of skill in the art would have been able to determine whether a particular mutant Fc-EPO falls within the scope of the claimed invention with only routine experimentation.

In summary, Applicants respectfully submits that the specification is sufficient to enable one of ordinary skill in the art to practice the claimed invention without undue experimentation because (1) the specification of the present invention provides adequate guidance or directions on how to practice the invention, (2) there was a high level of skill in the EPO art at the time when the application was filed, and (3) all of the methods needed to test EPO function and serum half-life were well known in the art. Accordingly, Applicants respectfully request the rejection be reconsidered and withdrawn.

## CONCLUSION

Claims 2-5, 16-18, 24, 25, and 27-40 are pending and presented for consideration. The Examiner is invited to telephone the undersigned attorney to discuss any remaining issues.

Respectfully submitted,



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Reg. No. 48,645

Brian Fairchild
Attorney for Applicant(s)
Testa, Hurwitz, & Thibeault, LLP
High Street Tower
125 High Street
Boston, Massachusetts 02110

Tel. No.: (617) 248-7697
Fax No.: (617) 248-7100

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